

Appl. No. 10/575,033  
Amdt. Dated December 9, 2011  
Reply to Office Action of June 9, 2011

Attorney Docket No. 374634-000078  
Customer No.: 73230

### **REMARKS**

Applicants wish to thank the Examiner for withdrawing previous rejections under 35 U.S.C. §102 and §103. Please reconsider the present application in view of the above amendments and the following remarks.

#### **Disposition of the claims**

Claims 1, 8 – 30 were previously pending. Claims 12 – 30 remain withdrawn. Independent claim 1 has been amended and dependent claim 10 has been canceled without prejudice. Accordingly, claims 1, 8, 9, and 11 are currently being examined on their merits.

#### **Amendment to the claims**

Independent claim 1 has been amended to recite that the transferrin domain (Tf) in the G-CSF-Tf fusion protein is loaded with at least one iron molecule. Support for this amendment can be found, for example, at least on page 11, line 10+ of the original specification and original claim 10. Accordingly, claim 10 has been canceled to avoid redundancy.

Applicants submit that no new matter has been introduced by this amendment.

#### **Rejections under 35 U.S.C. §102(b)**

Claims 1, 8 – 11 stand rejected under 35 U.S.C. §102(b) as being anticipated by Yeh et al. (US. Patent No. 5,665,863). Applicants note that claim 10 has been canceled, hence, this rejection is moot with respect to claim 10. To the extent that this rejection may still apply to the remaining claims, Applicants respectfully traverse.

Claim 1, as amended, recites:

A fusion polypeptide comprising a granulocyte colony stimulating factor (G-CSF) domain operably linked to a transferrin (Tf) domain, wherein the ability of the polypeptide to be transported into a cell expressing a transferrin receptor (TfR) gene or the ability of the polypeptide to be transported across a cell expressing a TfR gene via transcytosis is higher than that of the G-CSF domain alone, wherein the polypeptide is a recombinant polypeptide, and wherein said Tf domain **is preloaded with at least one iron molecule.**

The Examiner alleged that Yeh anticipates claim 1 because it teaches that one way to remedy the fast degradation rate of G-CSF is to stabilize it by making a chimera fusion protein of G-CSF with a protein possessing a long plasma half-life such as an albumin, an apolipoprotein, an immunoglobulin or alternatively a transferrin. The Examiner further asserted that because it was widely known in the art that transferrin binds to iron, the chimera construct of G-CSF-Tf(Fe) is therefore inherently or expressly anticipated. Applicants respectfully disagree.

MPEP §2131 states that “[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

MPEP §2121.01 also states that in order for a cited art document to anticipate a claim, the cited art must provide an enabling disclosure of the claimed subject matter. This section of the MPEP goes on to state that **the mere naming or description of the subject matter is insufficient**; rather, the cited art must demonstrate that the public was in possession of the claimed subject matter before the date invention. In other words, the cited art must describe the claimed subject matter in such detail as to enable one of ordinary skill in the art to make the claimed subject matter without undue experimentation.

In this instant case, Yeh fails to enable one of ordinary skill in the art to make and use the claimed subject matter without undue experimentation. For instance, claim 1 is drawn to a **recombinantly produced** chimera fusion protein capable of being transported into a cell expressing TfR, the chimera comprising a G-CSF domain linked to a Tf domain wherein the Tf domain is **preloaded with at least one iron** molecule. Yeh only teaches a method for extending plasma half-life of G-CSF by linking it to human serum albumin (HSA). Although Yeh theorized that transferrin is one possible protein structure that may be used as a stabilizing domain to extend the plasma half-life of G-CSF, Yeh failed to provide any teachings that may guide those skilled in the art to form a long plasma life G-CSF chimera other than HSA-G-CSF. The various proteins named by Yeh have widely different properties and the result of HSA chimera could not have been reasonably extrapolated to other chimera without undue experimentation. For example, serum albumin is unglycosylated whereas transferrin is glycosylated and lipoproteins are bound to lipids. The plasma half-life of these different classes of proteins will depend on the different biochemical pathways, which to a large part depend on certain unique features of their structures. Yeh is completely silent as to any common structural feature that actually enabled the long plasma half-life of these proteins, much less any teachings or suggestions that a Tf domain may be used to create a G-CSF-Tf chimera capable of being transported across cells expressing TfR. Without this knowledge, those skilled in the art could not have extrapolated the result of human serum albumin chimera to transferrin. In fact, Prior (US 7,176,279) specifically teaches that to extend plasma half-life of a Tf chimera, one should use a Tf mutant that is non-glycosylated and does not bind iron (col. 2, line 60+). Prior further states that as of August 30, 2001 (its earliest priority date), no Tf fusion protein has been created to extend the serum half-life of a therapeutic

protein or peptide (col. 2, line 39+). Because Yeh predated Prior by nearly 10 years, this is strong evidence that Yeh did not have in possession a sera-stable G-CSF-Tf chimera. At the very least, Yeh did not know that to extend the plasma half-life, both iron and carbohydrates needed to be removed from the Tf, which was the inventive discovery of Prior. Without this knowledge, Yeh's disclosure was clearly insufficient to enable those skilled in the art to make and use a sera-stable G-CSF-Tf chimera, much less a chimera capable of enhanced cell uptake in cells expressing the Tf receptor gene.

In view of the above, Applicants respectfully submit that Yeh fails to provide an enabling disclosure with respect to the claimed subject matter. Said another way, Yeh does not enable one of ordinary skill in the art to make and use the claimed subject matter without undue experimentation. As such, Applicants respectfully submit that the pending rejection is improper and that claim 1 is patentable over Yeh.

For at least the same reasons explained above, dependent claims 8, 9, and 11 are also patentable over Yeh. Accordingly, withdrawal of this rejection is respectfully requested.

**Rejection under 35 U.S.C. §103(a)**

Claims 1, 8 – 11 stand rejected under 35 U.S.C. §103(a) as being obvious in view of Yeh and Prior. As noted above, claim 10 has been canceled, hence, this rejection is moot with respect to claim 10. To the extent that this rejection may still apply to the remaining claims, Applicants respectfully traverse.

In the Office Action, the Examiner acknowledged that Yeh does not teach or suggest that the Tf domain should be loaded with iron. The Examiner further alleged that because Prior teaches that iron is needed for Tf to bind to Tf receptor in

order to cross the blood-brain barrier, it would have been *prima facie* obvious for those skilled in the art to make a G-CSF/Tf chimera and load it up with iron. Applicants disagree with the Examiner's analysis for at least the following reasons:

1. Lack of motivation

MPEP §2143.01(V) specifically states that the proposed modification cannot render the prior art unsatisfactory for its intended purpose. If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

In this instant case, the intended purpose of Yeh is to create a G-CSF chimera that will have longer plasma half-life. However, Yeh is completely silent as to how one might create such a chimera using transferrin.

On the other hand, Prior teaches using Tf as a sera stabilizing domain to extend plasma half-life of a therapeutic protein. Prior also teaches using Tf as a delivery vehicle for delivering nerve growth factor (NGF) or ciliary neurotrophic factor (CNTF) across the blood-brain barrier. It went on to explain that binding of two irons to the glycosylated Tf domain is required for Tf binding to its receptors on the target cell and to deliver NGF and CNTF across the blood-brain barrier. For extending plasma half-life, Prior emphasizes the need to prevent iron binding and glycosylation of the Tf domain. Taken as a whole, Prior makes clear that the effect of Tf in a chimera was not entirely predictable and that for extending sera half-life, a non-glycosylated mutant should be used.

Insofar as the intended purpose of Yeh is to create a sera-stable chimera, in this sense, Prior actually *teaches away* from preloading the Tf domain with iron.

In other words, because Prior teaches that Tf loaded with iron is good for crossing blood-brain barrier but not good for extending plasma half-life, and because there is no reason for G-CSF to be in the brain, at the time of the invention those skilled in the art contemplating making a G-CSF/Tf chimera would have considered using a Tf mutant without iron. There would have been no motivation or reasonable expectation of success for a G-CSF/Tf loaded with iron.

## 2. Unexpected results

Further, as explained in Applicants' specification, it was an unexpected discovery that a large protein such as G-CSF can actually be transported into GI cells when coupled with the Tf domain. This was a very surprising result. It eliminated the need to inject G-CSF and opened the door for oral administration formulations. Because there were already sera-stabilized G-CSF (e.g. the HSA-G-CSF chimera of Yeh, in which HSA has a plasma half-life of 15 – 20 days, see Exhibit A, 2<sup>nd</sup> sentence under the section USE OF HSA AS AN EXCIPIENT), there would have been no reason to create a less effective sera-stable form of G-CSF using transferrin (Tf plasma half-life is only 7 – 10 days, Prior Col. 2, line 13). That is, absent Applicants' disclosure, those skilled in the art would not have had any reasonable expectation of success to try a G-CSF/Tf chimera.

In light of the above, Applicants submit that claim 1 is patentable over Yeh and Prior. For at least the same reasons, claims 8, 9, and 11 are also patentable over Yeh and Prior.

Accordingly, withdrawal of this rejection is respectfully requested.

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In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles, California telephone number (310) 595-3107 to discuss the steps necessary for placing the application in condition for allowance.

If there are any fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 07-1896.

Date: December 9, 2011

Respectfully submitted,

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# Drug Delivery

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## Human Serum Albumin as a Pharmaceutical Excipient



### INTRODUCTION

Human serum albumin (HSA) is one of the most widely used and characterized proteins in the pharmaceutical field. It occurs naturally in the body, as a plasma protein, with a

concentration of 50 mg/mL. At this concentration, HSA regulates the colloidal osmotic pressure of blood. HSA is also responsible for transporting endogenous and exogenous compounds, which might be toxic in the unbound state, but non-toxic as albumin-bound. Human serum albumin purified from plasma is used for therapeutic applications, as a plasma expander, in situations involving severe blood loss. HSA is also widely used as an excipient, especially for biotechnology products. While the albumin used in marketed products is derived from plasma, recombinant versions of the protein are being investigated. Recombinant albumin can also alleviate any theoretical concerns of disease transmissivity associated with the human plasma-derived protein. This article provides a brief review of the use of albumin as a pharmaceutical excipient, and provides an update on the development of recombinant albumin.

### USE OF HSA AS AN EXCIPIENT

Human serum albumin is a 66-kD protein, with no glycosylation. The protein has molecular dimensions of 8 nm X 3.8 nm, and a half-life of 15 to 20 days. Due to its high concentration in plasma, HSA is not associated to significant extents with safety or immunogenicity concerns. A 5% albumin solution has an osmolarity of 265 mOsm/kg. Human serum albumin is a remarkably stable protein - it is the only therapeutic protein that is stable as a liquid at room temperature over the shelf life of the product. This is primarily due to the presence of 17 disulfide linkages present in the molecule. The intrinsic stability of the protein also allows it to be heated at 60°C for 10 hours to facilitate virus inactivation during manufacturing. This process has demonstrated elimination of both lipid-enveloped and certain non-lipid-enveloped viruses in validation experiments. The stability of albumin makes its storage and handling easier than typical proteins, thus lending itself well toward the use as an excipient.

Due to its established safety profile and unique properties, HSA is frequently used as a stabilizer for proteins. The protein has amphiphilic properties, which makes it suitable as an additive to inhibit

for proteins. The protein has amphiphilic properties, which makes it suitable as an additive to inhibit adsorption of the active protein to the container, via competitive adsorption mechanisms. The surface-active character of the protein also makes it suitable for use as a surfactant to prevent protein aggregation. HSA also has a high glass transition temperature, which in combination with its amphiphilic nature, makes it an ideal excipient for cryoprotection. For some proteins, the dual functionality (surfactant and cryoprotectant) results in better cryoprotection for albumin than disaccharides, as was observed by Liu, for Lactate dehydrogenase.<sup>1</sup> Table 1 lists representative commercial protein products that contain Albumin as an excipient.

Table 1. Products Utilizing Albumin as an Excipient		
Product	Level of Albumin	Company
Aralast (Alpha1 Proteinase Inhibitor)	5 mg/mL	Alpha Therapeutics, Baxter
Kogenate (Factor VIII)	10 mg/mL	Bayer
Intron A (Interferon alfa-2b)	1 mg/mL	Schering Plough
Avonex (Interferon beta-1)	16.5 mg	Biogen
Urokinase	250 mg	Abbott

Albumin is also being used as a carrier for microparticles and nanoparticles for sustained-release injectable drugs. A nanoparticulate formulation of paclitaxel containing albumin as the carrier was recently approved by the FDA. A number of researchers have also used albumin for sustained release of small molecules and proteins. Albumin's capacity to adsorb hydrophobic molecules makes it a unique carrier for controlled release because the drug gets released via desorption without significant burst effects. Albumin's adsorption capacity has also been exploited in development of magnetic microparticles. Such particles were used for targeted delivery of chemotherapeutic agents, such as doxorubicin. The particles consisted of albumin for binding of drug and iron for magnetic behavior to facilitate targeting.<sup>2</sup> Albumin microspheres have also been used in diagnostic applications to detect intravascular susceptibility.<sup>3</sup>

In recent years, albumin's long plasma circulation characteristics have been exploited to develop albumin-conjugated protein drugs that have longer half-lives as compared to the unconjugated protein. Albumin-fusion proteins are produced via recombinant techniques, and this concept has been used to extend the half-lives of a number of proteins including interferon- $\alpha$ <sup>4</sup>, interleukin-2, and G-CSF.<sup>5</sup>

## RECOMBINANT ALBUMIN

Although there has been no case of disease transmission for the use of HSA, a theoretical or perceived risk exists, due to which recombinant human albumin is currently being explored.<sup>6</sup> While this recombinant version is currently being explored as a therapeutic, its use as an excipient may be a logical progression, if the product gets approved.

A yeast-derived recombinant version was tested by Bosse and co-workers in a Phase I comparability study with human serum albumin.<sup>7</sup> The two proteins were compared side-by-side for both intravenous and intramuscular injections, involving more than 500 volunteers. No serious or potentially allergic events, or immunological response were reported with either product in the IV

potentially allergic events, or immunological response were reported with either product in the IV study. Serum albumin, colloid osmotic pressure changes, and hematocrit ratio were also similar. The authors concluded that rHA and HSA exhibited similar safety, tolerability, and pharmacokinetic/pharmacodynamic profiles, with no evidence of any immunological response. Tarelli and co-workers investigated the use of recombinant albumin as a cryoprotectant for thyroid-stimulating hormone (TSH), interleukin 15 (IL-15), and granulocyte colony-stimulating factor (G-CSF).<sup>8</sup> It was observed that the recombinant albumin was equivalent in its functionality to HSA, for stabilization of the proteins as well as binding of fatty acids.

**Table 2. Companies Involved in Recombinant Albumin Development**

Company	Product Name	Technology	Status
Delta Biotechnology	Recombumin	Recombinant (Yeast derived)	Type V Biologics Master File has been submitted
GTC Biotherapeutics	RHA	Transgenics	In Development
Mitsubishi Welfarma	Recombinant	Alburex	Approved in Japan

## SUMMARY

Albumin is a well characterized protein and serves important needs as a therapeutic, diagnostic agent, as well as an excipient. While use of albumin as an excipient has met some resistance due to perceived risk of disease transmission, recombinant albumin is being developed to address any such concerns. Recombinant albumin may also serve as a useful case study for follow-on biologics.<sup>9</sup> However, use of recombinant albumin as an excipient, would depend on the efficiency of the manufacturing process, to allow for reasonable cost of goods.

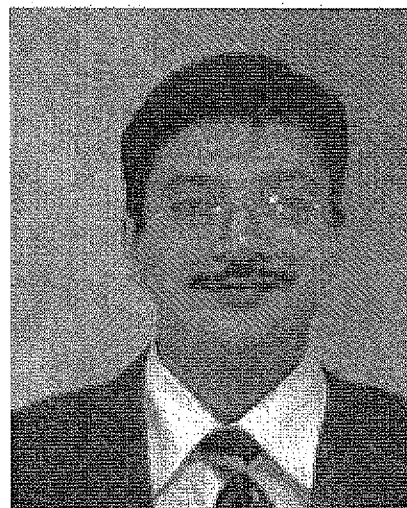
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## BIOGRAPHY

**Dr. Mahesh V. Chaubal** is an Associate Director in the Scientific Affairs division at Baxter Healthcare. Dr. Chaubal earned his BS in Chemical Engineering from the University of Bombay (UDCT) and his MS and PhD in Chemical and Biochemical Engineering from the University of Maryland. His PhD research involved developing novel formulations for protein C, a zymogen precursor of the active ingredient of Xigris, which is the first FDA-approved protein therapeutic for severe sepsis. Dr. Chaubal has worked in the field of drug delivery and pharmaceutical polymers for more than 10 years and has published more than 50 peer-reviewed articles, symposium abstracts, and industry reports in this field. He was a member of the team that brought Gliadel®,



the first sustained-release chemotherapeutic formulation, to market. His experience in drug delivery systems includes novel drug delivery research, development of innovative formulations for water-insoluble drugs, proteins and gene-based drugs, as well as scale-up and validation of processes for drug delivery formulation processes. Dr. Chaubal is also the Founder of Drugdel.com, an online information website specializing in the field of drug delivery and alternative formulations.